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Delayed return to estrus following treatment with the gonadotrophin-releasing hormone agonist, Lucrin® Depot, in the tammar wallaby

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ABSTRACT

Gonadotrophin-releasing hormone agonists that induce a reversible contraceptive state in several marsupials have the potential to be used to synchronize estrus. We used a model macropod, the tammar wallaby (*Notamacropus eugenii*), to investigate whether Lucrin Depot (AbbVie), a GnRH agonist microsphere preparation, could (1) inhibit follicular development and estrus in a cycle reactivated by removal of pouch young (RPY) and (2) facilitate a synchronous return to estrus. Our results show that females reactivated with bromocriptine and RPY in early seasonal quiescence (July 2015) were inhibited by Lucrin Depot (0.125–0.5 mg kg $^{-1}$, $n = 9$) and unlike control females ($n = 3$), did not copulate before Day 32 RPY. During the next breeding season (February 2016), the return to estrus after RPY was not delayed in animals treated with Lucrin Depot ($\leq$0.20 mg kg $^{-1}$; $n = 12$), and copulation occurred in treated and control females within the expected natural period after RPY (Day 26–33 RPY). In the following breeding season (March 2017), estrus was delayed in animals treated with Lucrin Depot (1.25 mg kg $^{-1}$) on either Day 0 (Group A, $n = 6$) or Day 10 (Group B, $n = 6$) after RPY compared to control females ($n = 6$). Estrus was detected in Group A between 39 and 66 days (55 ± 4.8d) and in Group B between 43 and 71 days (55.2 ± 3.9d) after RPY. In contrast, all control females underwent estrus and copulated as expected by Day 30 RPY. We conclude Lucrin Depot can inhibit ovarian follicular activity after RPY but as a standalone treatment does not result in a highly synchronous return to estrus in the tammar wallaby.

1. Introduction

Female synchronization technology remains a priority research area for the development of assisted breeding technologies for marsupials, and is expected to revolutionize conservation programs for threatened species by supporting the use of genetically diverse frozen-thawed, chilled or fresh semen in artificial insemination [1,2]. However, estrous synchronization methods effective in eutherian mammals, that are based on prostaglandins and exogenous progesterone which either extend or disturb the lifespan of the corpora lutea (CL), are not effective in marsupials [1,3]. Such methods have failed due to an inability to manipulate the lifespan of the marsupial CL in which, unlike the eutherian CL, once formed becomes independent of hypothalamic-pituitary support [4–9]. Despite this feature of ovarian function, marsupial reproduction, as in eutherian mammals, is controlled by the release of gonadotrophin releasing hormone (GnRH) from the hypothalamus in intermittent pulses which regulates the rate that follicle stimulating hormone (FSH) and luteinizing hormone (LH) is secreted by the anterior pituitary [5]. FSH stimulates follicle growth and development and LH supports the final stages of follicle maturation and formation of the CL [5].

Deslorelin, a GnRH agonist implant, induces a reversible contraceptive state in a range of marsupials by down-regulation of the hypothalamic-pituitary-gonadal (HPG) axis [10–15]. These results suggested that GnRH analogues may have potential as agents for female synchronization. Two GnRH analogues have been trialed for synchronization in the koala (*Phascolarctos cinereus*) with variable results. The GnRH agonist buserelin (4 μg i.m.) induced a return to estrus in 3 of 5 koalas, but the remaining two females did not return to estrus within the breeding season [16]. Likewise, the GnRH antagonist azaline B (1 mg s.c. for 10 days) successfully inhibited the LH response, folliculogenesis and estrus in the koala, but did not result in a synchronous return to estrus (range 9–39 days) [17]. Lucrin Depot (AbbVie), a long-acting GnRH agonist microsphere

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preparation of leuprorelin acetate, has shown considerable promise as an estrous control agent for marsupials [18,19]. In the female fat-tailed dunnart (*Sminthopsis crassicaudata*), a single injection of either 5 or 10 mg kg $^{-1}$ inhibited estrous cycles for 4–8 weeks [18,19], some females commenced cycling between 8 and 12 weeks, and all females were cycling normally by 16 weeks after treatment [19]. Further, female dunnarts were able to conceive and give birth to healthy pouch young. A retrospective calculation of conception indicated that two of six females treated with 5 mg kg $^{-1}$ gave birth to young conceived at 8 weeks after treatment, while embryos recovered from two of six females treated with 10 mg kg $^{-1}$ at 16 weeks after treatment were estimated to have conceived in week 14 and 15 [19]. The response to treatment with Lucrin Depot in the dunnart is similar to that observed in eutherian mammals. In rats and dogs Lucrin Depot suppressed the estrous cycle temporarily for a 6-week period, and normal reproductive function returned at about 8–10 weeks after treatment [20]. The advantage of Lucrin Depot compared with other GnRH agonists is the polylactic/glycolic acid (PLGA) polymer microsphere preparation which facilitates a linear release of leuprorelin over four weeks [20]. Lucrin Depot, with its highly tailored release properties, could have the capacity to 'reset' cycling in female marsupials allowing for a synchronous return to estrus as the microspheres deplete.

This study builds on the assessment of the effects of Lucrin Depot for control of estrus in the fat-tailed dunnart [18,19] by examining its potential for synchronization of estrus in the female tammar wallaby (*Notamacropus eugenii:* formerly *Macropus eugenii* [21,22]). The tammar wallaby, is a monovular, polyestrous and seasonal breeder in which ovulation occurs in the alternate ovary in each successive cycle [23,24]. The tammar is an established experimental model which has been the subject of earlier studies focused on the development of reproductive technology [25–30] including deslorelin-induced contraception [10,11,31]. The tammar has a highly predictable and regimented reproductive strategy [5,9,32–35] (Fig. 1). The breeding season begins after the summer solstice and continues to the winter solstice (lactational quiescence). The non-breeding season (seasonal quiescence) follows [7,32,36–38]. In the Southern Hemisphere, the first births of the year occur between late January and early February. Birth is followed by a post-partum estrus, mating, ovulation and conception. The newly formed CL and blastocyst are both inhibited in embryonic diapause by the sucking stimulus of the pouch young (lactational quiescence) [7,32]. During lactational quiescence, the loss or removal of the pouch young (RPY) results in reactivation of the quiescent CL and the diapausing blastocyst. For the first 19 days after RPY, follicle growth is inhibited by the CL [25], after which the follicular phase proceeds for 8–10 days [39]. Birth occurs on Day 26–27 RPY [23,32,36,37]. The female enters post-partum estrus and copulates with a male within about 6 h [23,40,41]. The Graafian follicle ovulates approximately 24–30 h after birth and mating [23]. In the non-pregnant female, RPY also reactivates the CL, and estrus and mating follow 30.4 ± 0.99 days later [37]. In the non-breeding season, embryonic diapause is maintained by increasing day-length as well as the suckling stimulus, and RPY does not reactivate the CL and blastocyst [7,32]. However, experimental reactivation of the CL can be extended until September (into the period of seasonal quiescence after the winter solstice) by combining RPY with bromocriptine mesylate treatment [34] (Fig. 1).

Here, we have investigated the potential for Lucrin Depot to be used as a female synchronization agent in a macropod marsupial, using the female tammar wallaby as a model. There were two objectives: (1) to determine the dose of Lucrin Depot required to inhibit estrus in the first cycle following RPY, and (2) to establish if the timing of a subsequent return to estrus is synchronous among females.

2. Materials and methods

2.1. Animals

The tammar wallabies (*Notamacropus eugenii*) were sourced from the CSIRO wallaby breeding colony, originally derived from Kangaroo Island, held at CSIRO, Crace, ACT. The tammars were held in a ratio of three females to one male in open grassy triangular pens (7.5 m × 18.5 m x 18m). Ewe and Lamb cereal pellets (Quayle Milling Quality Livestock Nutrition, Young, NSW) and water were available *ad libitum*. All females were adult (>2years old) and were carrying a pouch young at the start of an experiment.

These experiments were approved by the CSIRO Wildlife and Large Animal Ethics Committee (AEC project no. 2015-12).

2.2. Experiment 1: effect of Lucrin Depot dose in tammars reactivated during early seasonal quiescence: preliminary study

This pilot experiment aimed to define a dose of Lucrin which would inhibit follicular activity in the tammar wallaby. The study was conducted in late July 2015 (early seasonal quiescence/non-breeding season). Four groups of 3 animals were randomly assigned to a treatment (0, 0.125, 0.25 or 0.50 mg kg $^{-1}$ Lucrin Depot) at the time of RPY (Day 0). To ensure reactivation of the

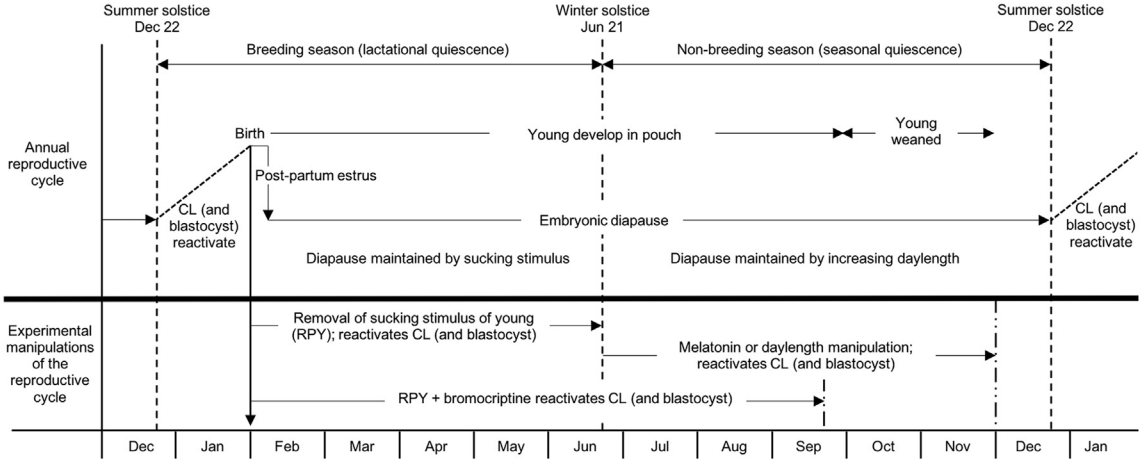

Fig. 1. The annual reproductive cycle of the tammar wallaby (*Notamacropus eugenii*) and experimental manipulations of the cycle.